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GENETIC DIVERSITY OF *PANAX VIETNAMENSIS* VAR. *FUSCIDISCUS* K. KOMATSU, S. ZHU & S.Q. CAI POPULATION IN WESTERN NORTH OF VIETNAM DETECTED BY INTER SIMPLE SEQUENCE REPEAT MARKERS

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SUMMARY

Panax vietnamensis var. *fuscidiscus* is a precious medicinal plant which was recently discovered in Sin Ho and Muong Te district, Lai Chau province of Vietnam. Over exploitation of the species in the native habitat poses a serious threat to its existence. Adequate information on the nature and the extent of genetic diversity in this important species is required for developing suitable strategy for its conservation. In this study, inter simple sequence repeat markers were employed to investigate the genetic diversity and variability of 46 individuals belonging to a naturally distributed population of this variety in Vietnam. Genetic diversity at the population level was high (H_e = 0.2300, I = 0.3665, and PPB = 96.98 %). The group of mature individuals possessed the higher genetic diversity (H_{eO} = 0.2291, I_O = 0.3563, and PPB_O = 84.34 %) as compared to group of young individuals (H_{eY} = 0.2086, I_y = 0.3291, and PPB_Y = 81.5 %). The intergroup gene differentiation was high (G_{ST} = 0.0499) with the genetic distance among groups was 0.0298. The similarity coefficient among mature individuals was more moderate (Maximum = 0.873, Minimum = 0.614 and Average = 0.741) than among young individuals (Maximum = 0.916, Minimum = 0.596 and Average = 0.759). Otherwise, the number of discovered individuals was small, distribution area is narrow habitats, and the population showed the reduction in genetic diversity due to the human affects in the habitat and over-exploitation. Results on genetic diversity and variability showed that the investigated population has coped with the risk of decline and needed to be protected.

Keywords: *Genetic diversity, inter simple sequence repeat, Panax vietnamensis* var. *fuscidiscus, Vietnam*

INTRODUCTION

Panax L. (ginseng) is a small genus of Araliaceae, which is distinguished from other genera of Araliaceae by slow-growing perennial plants with fleshy roots, stout rootstock. They are the most famous and valuable medicinal plants in the world. In 2003, Zhu *et al.* described a new variety of *Panax vietnamensis* var. *vietnamensis* and named as *Panax vietnamensis* var. *fuscidiscus*. This variety was discovered in Jinping county of Yunnan province, China. In Vietnam, *P. vietnamensis* var. *fuscidiscus* (Lai Chau ginseng) was naturally distributed in Sin Ho district, Lai Chau province, Western North of Vietnam (Phan *et al.*, 2013).

Lai Chau ginseng is found in higher elevations, between 1600 and 2000 m. The investigated population grows in small groups scattered amongst the herbaceous storey of primary, closed, evergreen, seasonal, tropical, broad-leaved forests on sandy and shale soils (wet and well-drained). This population is very well adapted to the tropical monsoon climate associated with these particular mountainous localities. In the investigated population, mature individuals have been harvested and used by the indigenous people as material for some medical treatments and health enhancement for centuries. And, recently rhizomes of Lai Chau ginseng have been traded as one of *P. vietnamensis* var. *vietnamensis* (Ngoc Linh ginseng) adulterants, this increases the harvesting pressure on Lai Chau ginseng. Overexploitation of these medicinal plant's rhizomes for medical uses has led to the loss of

genetic biodiversity and as a result the studied species was classified as critically endangered (CR) under the national category with criteria of A2,b,c,d; B2b(ii,iii,v); C2a(i); E (Phan *et al.*, 2013).

Due to the limited distribution, only 80-100 individuals were found in their natural habitat during field investigations in the period 2013 and 2014. Studies on the genetic diversity of this variety have not been conducted in Vietnam. Reduction in genetic diversity is actual risk to *P. vietnamensis* var. *fuscidiscus*. Genetic variation is currently understood as a critical variable to the long-term survival of a population or species (Beardmore, 1983; Anatonovis, 1984). Understanding the genetic diversity and variation within and among groups/subpopulations of population of rare and endangered taxa is essential when developing management strategies for both *in situ* and *ex situ* conservation activities (Hogbin, Peakall, 1999). Thus, estimating inter- and intra-groups/subpopulations genetic diversity is critical to the protection and long-term availability of *P. vietnamensis* var. *fuscidiscus* in both terms of ecological biodiversity and medically-related uses. Current research methods support the use of molecular markers as suitable and accurate tools for population genetic diversity detection. The advantages of inter simple sequence repeat (ISSR) lies within its low-cost use, convenience of use, and high-level of reliability in reproducing results (Zietkiewicz *et al.*, 1994; Nagoaka, Ogihara, 1997; Lu *et al.*, 2009; Roy, Chakraborty, 2009). As such, ISSR methods have established wide spread and accepted use for applications in population genetic studies of both wild and cultivated plants (Roy, Chakraborty, 2009).

In the current study, the ISSR marker system was employed to induce DNA fingerprints for the estimation of genetic diversity of wild *P. vietnamensis* var. *fuscidiscus* population in Lai Chau, Vietnam and investigation of genetic diversity and differentiation in its mature and young groups which distributed in the same natural habitats. The objectives of this study were as follows: (1) to estimate genetic diversity at population and age group levels; (2) to analyze genetic relationships and differentiation among mature and young groups belonging to the population, and (3) to contribute and catalogue the data of this study for the use in the conservation and sustainable utilization of the researched medicinal plants within Vietnam.

MATERIALS AND METHODS

Plant materials

From March 2013 to May 2014, a total of 46 individuals presenting naturally distributive population of the *P. vietnameensis* var. *fuscidiscus* from Lai Chau Province, Vietnam which corresponded to 2 groups: young and mature, were sampled across their original habitat (Table 1). Twenty four young individuals (≤3-4 years) and 22 mature individuals (> 6-10 years) were randomly selected for DNA extraction. Chosen individuals for sampling were separated from each other at least 50 m.

Fresh leaves were collected, kept fresh if DNA extraction within 60 hours or dried in sealed bags with silica gel if DNA extraction executed over 60 hours later and brought to the laboratory where each sample was extracted and preserved at a constant -20^0 C for DNA analysis.

Table 1. Geographic localities of *P. vietnamensis* var. *fuscidiscus* populations in this study. O: belong to mature group; Y: belong to young group; x: number for sample identification.

Sample quantity and sign	Age group	Geographic localities	Longitude/Latitude range
22 samples, signed as Ox	> 6 -10 years	Muong Te district and Sin Ho district	$102^0$48'35'' to $103^0$14'02'' E
24 samples, signed as Yx	≤ 3-4 years		$22^0$13'53' to $22^0$30'51''N

DNA extraction purification and quantification

Total genomic DNA was extracted using Cetyltrimethyl Ammonium Bromide (CTAB) protocol I (Weising *et al*., 2007) with a modification of adding 10 % SDS to the extraction buffer which was then dissolved in water for the subsequent use. The DNA concentration (C) was calculated as follows: C (µg /µL) = OD260 × 50. The OD 260/ 280 ratio was also calculated to determine DNA purity (Weising *et al.*, 2007).

ISSR-PCR amplification

ISSR primers used in this study were synthesized by Bioneer Corporation (Republic of Korea), according to the primer set published by the University of British Columbia and Zagazig University (Egypt). Sixty ISSR primers were initially screened, and 17 of them, which yielded bright, clear bands and at least possessed one polymorphic band in both populations, were used for the analysis of all 60 samples (Table 2). PCR amplification was repeated for those working primers to check the stability and reproducibility of ISSR DNA fingerprinting. PCRs were performed in 20 µl reactions containing 2 mmol/L $MgCl_2$, 0.25 mmol/L each of dNTPs, 1U Taq DNA polymerase (ThermoScientific), 0.2 µmol/L primer and approximately 30 ng DNA templates. The amplifications were performed in a Peqstar 96X Universal Gradient thermocycler (PEQLAB Biotechnologie GmbH, Germany) with the following program: initial denaturation at 94 ^{0}C for 5 min; 10 cycles of 94 ^{0}C for 45 s, annealing temperature +5 (T_a +5) ^{0}C (Table 2) for 45 s, decreased 0.5 ^{0}C/cycle, 72 ^{0}C for 1 min 30 s; 36 cycles of 94 ^{0}C for 45 s, annealing temperature for 45 s, 72 ^{0}C for 1 min 30 s; Final extension at 72 ^{0}C for 15 min; the amplification products were separated in 2 % agarose gel, using TBE buffer at 60 V for 3 hours, stained with ethidium bromide (0.5 µg/ml), and photographed under 254/312 nm wavelength lights using Micro Doc Gel Documentation System (Cleaver Scientific, USA).

Data analysis

Since ISSR markers were dominantly inherited, each band was assumed to represent the phenotype at a single biallelic locus (Williams *et al*., 1990). ISSR bands were scored as presence (1) or absence (0) characters, to construct the binary data matrix.

POPGENE software (1.32) was used to calculate genetic diversity parameters: the percentage of polymorphic bands (*PPB*), the average expected heterozygosity (H_e), average effective number of alleles (*ne*), the gene differentiation (G_{ST}), the genetic distance among investigated sample sets (*D*) and (Yeh *et al*., 1997). The Nei's genetic distance between pair of sample sets is calculated as: $D_{XY} = -\ln(I_{XY})$ is based on the concept of genetic identity (I_{XY}): $I_{XY} = J_{yo}/\sqrt{(J_X \times I_Y)}$, where: J_X = average homozygosity in the first sample set, J_Y= average homozygosity in the second sample set, J is calculated by formula J=1-H_{eT}, J_{XY}= average inter-sample set homozygosity = j_{XY}/L with j_{XY} is homozygosity among two sample sets and L is the number of investigated loci. $J_{XY} = \Sigma_{XYjk}(p_{XjK} \times p_{YjK})$, where p_{XjK} and p_{YjK} are frequencies of K^{th} allele at locus j^{th} in the first and the second sample sets, respectively (Vicente *et al.*, 2003).

Similarity coefficient between pair of samples and UPGMA dendrogram for genetic relationship among all studied samples was calculated and established by using NTSYSpc 2.1 (Numerical Taxonomy and Multivariate Analysis System) software (Rohlf, 2004).

RESULTS

Genetic diversity

The twenty selected primers yielded 166 reproducible bands for total investigated samples. For population level, the number of bands per primer varied between 5 (UBC856C, HB11) and 15 (HB12), with an average of 8.3. For whole population, the *PPB* per primer varied from 83.33 % (ISSR844A, 17899A) to 85.71 % (ISSR 814, 17899B), 87.50 % (UBC807) and 100% (all remaining primers). For young individual group, the *PPB* per primer varied from 42.86 % (17899B) to 100 % (ISSR808, HB8, HB12, HB15, UBC842, UBC856T, UBC873). For mature individual group, the *PPB* per primer varied from 60 % (UBC856T) to 100 % (HB11, UBC842C). There was the reduction of *PPB* from population to age group levels (Table 2).

In the investigated population, the expected genetic heterozygousity was H_e = 0.230, Shannon information index I = 0.3665, the percentage of polymorphic bands was *PPB* = 96.98 %, average effective number of alleles *Ne* = 1.7342. Among the two individual groups separated by age, young group possessed the lower level of genetic diversity (H_{eY} = 0.2086, I_Y = 0.3291, PPB_Y = 81.5 % and Ne_Y = 1.3356), while mature group harbored higher level (H_{eO} = 0.2291, I_O = 0.3563, PPB_O = 84.34 % and Ne_O = 1.8434).

Genetic relationship

In young group, the genetic similarity coefficients among the individuals ranged from 59.96 % ($Y_{10} - Y_{11}$) to 91.60 % ($Y_{14} - Y_{15}$) with a mean of 75.90%, while those of mature group ranged from 61.40 % ($O_1 - O_7$) to 87.30 % ($O_{13} - O_{14}$) with a mean of 74.41 (Table 3 & 4). The gene similarity

coefficients among the individuals of population were varied, ranging from 59.00 % (Y_8 – O_{20}) to 91.60 % (Y_{14} – Y_{15}) with a mean of 74.00 % (Table 3, 4, 5). The intergroup gene differentiation among two age groups was G_{ST} = 0.0499 with the genetic distance between them was D = 0.0298.

Table 2. ISSR primers used in this study.

Primer code	Sequence (5' →3')	T_a (^{0}C)	Total recorded bands for whole population	*PPB* (%)		
				Whole population	Young group	Mature group
808	$(AG)_8$ C	52	9	100.00	100.00	88.89
814	$(CT)_8$ TG	51,5	7	85.71	85.71	85.71
844A	$(CT)_8$ AC	52	6	83.33	83.33	83.33
17898A	$(CA)_6$ AC	54,5	10	100.00	90.00	90.00
17898B	$(CA)_6$ GT	54,5	8	100.00	87.50	87.50
17899A	$(CA)_6$ AG	54	6	83.33	66.67	66.67
17899B	$(CA)_6$ GG	54	7	85.71	42.86	71.43
HB8	$(GA)_6$ GG	52	9	100.00	100.00	66.67
HB9	$(GT)_6$ GG	52	8	100.00	62.50	87.50
HB10	$(GA)_6$ CC	52	13	100.00	61.54	84.62
HB11	$(GT)_6$ CC	52	5	100.00	60.00	100.00
HB12	$(CAC)_3$ GC	52	15	100.00	100.00	86.67
HB15	$(GTG)_3$ GC	52	11	100.00	100.00	90.91
UBC 807	(AG)8 T	54	8	87.50	50.00	87.50
UBC 826	$(AC)_8$ C	54	7	100.00	85.71	85.71
UBC 842C	$(GA)_8$ CG	51,5	7	100.00	100.00	100.00
UBC 842T	$(GA)_8$ TG	51,5	10	100.00	90.00	90.00
UBC 856C	$(AC)_8$ CA	52	7	100.00	85.71	71.43
UBC 856T	$(AC)_8$ TA	52	5	100.00	100.00	60.00
UBC 873	$(GACA)_4$	52	8	100.00	100.00	87.50
Total			166			
Average			8.3	96.98	83.73	84.34

Table 3. Genetic similarity coefficients among the individuals belong to young group

	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15	Y16	Y17	Y18	Y19	Y20	Y21	Y22	Y23
Y2	0.801																						
Y3	0.783	0.777																					
Y4	0.741	0.663	0.693																				
Y5	0.777	0.687	0.741	0.735																			
Y6	0.801	0.723	0.789	0.783	0.819																		
Y7	0.789	0.735	0.801	0.699	0.807	0.867																	
Y8	0.717	0.747	0.801	0.651	0.675	0.699	0.747																
Y9	0.723	0.693	0.711	0.608	0.777	0.741	0.837	0.681															
Y10	0.699	0.657	0.663	0.596	0.753	0.765	0.801	0.633	0.855														
Y11	0.681	0.614	0.669	0.699	0.651	0.699	0.699	0.614	0.620	0.596													
Y12	0.783	0.741	0.783	0.693	0.789	0.825	0.837	0.693	0.831	0.771	0.705												
Y13	0.783	0.717	0.759	0.657	0.801	0.801	0.873	0.657	0.819	0.795	0.669	0.855											
Y14	0.765	0.711	0.717	0.711	0.843	0.819	0.807	0.639	0.801	0.813	0.651	0.801	0.825										
Y15	0.789	0.723	0.741	0.735	0.855	0.807	0.831	0.663	0.801	0.789	0.711	0.825	0.849	0.916									
Y16	0.801	0.759	0.753	0.723	0.771	0.795	0.795	0.687	0.729	0.741	0.747	0.777	0.813	0.819	0.855								
Y17	0.741	0.747	0.789	0.735	0.759	0.795	0.759	0.723	0.729	0.693	0.663	0.789	0.777	0.759	0.795	0.759							
Y18	0.807	0.753	0.783	0.693	0.729	0.753	0.753	0.729	0.675	0.675	0.681	0.771	0.759	0.705	0.789	0.801	0.789						
Y19	0.765	0.795	0.789	0.675	0.771	0.807	0.819	0.735	0.789	0.777	0.687	0.837	0.813	0.747	0.783	0.819	0.783	0.801					
Y20	0.771	0.837	0.771	0.693	0.693	0.801	0.777	0.765	0.711	0.675	0.633	0.795	0.771	0.741	0.729	0.801	0.789	0.747	0.825				
Y21	0.831	0.753	0.831	0.753	0.789	0.801	0.813	0.765	0.735	0.687	0.693	0.795	0.759	0.717	0.777	0.777	0.789	0.819	0.825	0.759			
Y22	0.783	0.789	0.795	0.657	0.717	0.753	0.789	0.729	0.747	0.723	0.717	0.807	0.783	0.693	0.765	0.825	0.765	0.807	0.825	0.795	0.843		
Y23	0.777	0.795	0.765	0.699	0.711	0.771	0.747	0.723	0.705	0.681	0.747	0.789	0.741	0.699	0.771	0.819	0.759	0.777	0.807	0.813	0.801	0.910	
Y24	0.807	0.729	0.771	0.765	0.837	0.801	0.825	0.681	0.771	0.735	0.693	0.819	0.807	0.825	0.886	0.789	0.813	0.795	0.789	0.759	0.831	0.795	0.789

Table 4. Genetic similarity coefficients among the individuals belong to mature group.

	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	O14	O15	O16	O17	O18	O19	O20	O21
O2	0.675																				
O3	0.693	0.837																			
O4	0.723	0.711	0.813																		
O5	0.645	0.729	0.759	0.753																	
O6	0.759	0.723	0.741	0.735	0.681																
O7	0.614	0.735	0.717	0.675	0.825	0.663															
O8	0.669	0.741	0.723	0.729	0.723	0.705	0.693														
O9	0.747	0.795	0.813	0.795	0.753	0.747	0.723	0.741													
O10	0.717	0.777	0.747	0.729	0.747	0.765	0.729	0.735	0.849												
O11	0.735	0.759	0.777	0.819	0.765	0.723	0.699	0.789	0.795	0.753											
O12	0.645	0.717	0.735	0.693	0.687	0.681	0.645	0.687	0.753	0.687	0.717										
O13	0.693	0.753	0.759	0.777	0.699	0.741	0.705	0.723	0.789	0.747	0.753	0.735									
O14	0.735	0.747	0.765	0.771	0.705	0.735	0.711	0.741	0.795	0.717	0.771	0.693	0.873								
O15	0.705	0.729	0.783	0.789	0.699	0.765	0.681	0.735	0.765	0.747	0.813	0.699	0.783	0.789							
O16	0.747	0.723	0.765	0.807	0.669	0.783	0.663	0.717	0.783	0.777	0.783	0.765	0.789	0.771	0.777						
O17	0.657	0.789	0.795	0.777	0.759	0.741	0.753	0.747	0.801	0.771	0.777	0.735	0.807	0.801	0.783	0.753					
O18	0.687	0.795	0.741	0.687	0.729	0.723	0.699	0.741	0.783	0.825	0.747	0.729	0.753	0.735	0.741	0.711	0.777				
O19	0.717	0.729	0.759	0.789	0.711	0.765	0.717	0.711	0.777	0.783	0.753	0.759	0.783	0.765	0.795	0.801	0.771	0.777			
O20	0.633	0.717	0.771	0.741	0.711	0.693	0.693	0.675	0.729	0.687	0.717	0.747	0.771	0.741	0.747	0.705	0.759	0.705	0.783		
O21	0.693	0.753	0.759	0.741	0.687	0.741	0.657	0.735	0.777	0.819	0.789	0.687	0.735	0.717	0.735	0.741	0.723	0.765	0.771	0.711	
O22	0.699	0.735	0.777	0.771	0.717	0.699	0.651	0.717	0.771	0.693	0.795	0.705	0.789	0.795	0.765	0.735	0.729	0.699	0.693	0.741	0.717

Table 5. Genetic similarity coefficients between the individuals from young and mature group.

	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15	Y16	Y17	Y18	Y19	Y20	Y21	Y22	Y23	Y24
O1	0.687	0.681	0.747	0.705	0.681	0.729	0.705	0.717	0.639	0.639	0.633	0.699	0.663	0.657	0.705	0.705	0.705	0.759	0.729	0.687	0.759	0.711	0.705	0.675
O2	0.783	0.729	0.771	0.717	0.801	0.777	0.789	0.693	0.771	0.723	0.729	0.807	0.783	0.765	0.813	0.789	0.765	0.747	0.765	0.747	0.831	0.771	0.753	0.819
O3	0.801	0.687	0.741	0.723	0.771	0.759	0.771	0.699	0.705	0.681	0.747	0.741	0.765	0.723	0.771	0.795	0.723	0.765	0.783	0.729	0.837	0.777	0.747	0.801
O4	0.783	0.681	0.747	0.741	0.765	0.741	0.729	0.681	0.651	0.602	0.729	0.711	0.723	0.681	0.741	0.741	0.705	0.783	0.717	0.699	0.783	0.747	0.729	0.759
O5	0.753	0.699	0.681	0.675	0.831	0.771	0.747	0.590	0.741	0.729	0.651	0.765	0.777	0.855	0.819	0.723	0.711	0.693	0.723	0.681	0.717	0.669	0.663	0.789
O6	0.759	0.765	0.771	0.717	0.693	0.777	0.741	0.729	0.687	0.651	0.633	0.735	0.687	0.693	0.681	0.705	0.741	0.747	0.729	0.783	0.795	0.735	0.729	0.735
O7	0.711	0.633	0.675	0.669	0.789	0.753	0.741	0.596	0.759	0.795	0.633	0.759	0.771	0.873	0.825	0.729	0.717	0.663	0.705	0.675	0.699	0.651	0.657	0.771
O8	0.741	0.687	0.693	0.687	0.735	0.723	0.711	0.590	0.657	0.645	0.663	0.681	0.717	0.735	0.747	0.771	0.687	0.693	0.687	0.705	0.729	0.705	0.699	0.765
O9	0.819	0.717	0.759	0.753	0.801	0.837	0.801	0.669	0.687	0.699	0.753	0.771	0.795	0.777	0.837	0.849	0.765	0.831	0.789	0.735	0.831	0.795	0.801	0.819
O10	0.777	0.759	0.765	0.687	0.771	0.831	0.831	0.711	0.765	0.753	0.675	0.813	0.801	0.807	0.819	0.819	0.747	0.789	0.807	0.801	0.789	0.789	0.795	0.825
O11	0.819	0.705	0.723	0.753	0.765	0.777	0.741	0.681	0.711	0.687	0.729	0.735	0.747	0.765	0.813	0.789	0.741	0.771	0.741	0.711	0.795	0.735	0.753	0.759
O12	0.717	0.627	0.608	0.723	0.699	0.723	0.675	0.602	0.620	0.620	0.687	0.657	0.645	0.687	0.699	0.687	0.687	0.657	0.651	0.681	0.717	0.657	0.675	0.729
O13	0.777	0.687	0.741	0.771	0.759	0.771	0.711	0.639	0.633	0.645	0.747	0.717	0.693	0.711	0.747	0.759	0.759	0.753	0.687	0.705	0.753	0.705	0.759	0.813
O14	0.759	0.693	0.699	0.801	0.753	0.753	0.705	0.608	0.627	0.651	0.741	0.723	0.711	0.693	0.753	0.765	0.741	0.735	0.693	0.699	0.771	0.735	0.753	0.795
O15	0.813	0.687	0.741	0.747	0.699	0.735	0.735	0.675	0.681	0.657	0.723	0.765	0.705	0.699	0.759	0.747	0.699	0.765	0.711	0.693	0.801	0.741	0.735	0.753
O16	0.759	0.693	0.723	0.777	0.705	0.753	0.741	0.753	0.639	0.614	0.705	0.723	0.675	0.681	0.729	0.717	0.729	0.747	0.717	0.747	0.819	0.723	0.765	0.807
O17	0.741	0.663	0.681	0.747	0.759	0.807	0.747	0.590	0.705	0.705	0.735	0.741	0.729	0.759	0.783	0.747	0.723	0.729	0.687	0.705	0.777	0.681	0.711	0.789
O18	0.759	0.717	0.723	0.693	0.729	0.789	0.789	0.620	0.747	0.747	0.669	0.819	0.795	0.777	0.765	0.753	0.717	0.735	0.753	0.735	0.735	0.723	0.717	0.759
O19	0.741	0.711	0.741	0.735	0.711	0.747	0.771	0.687	0.681	0.645	0.735	0.765	0.729	0.723	0.759	0.771	0.735	0.753	0.723	0.729	0.789	0.753	0.783	0.765
O20	0.705	0.639	0.645	0.735	0.675	0.699	0.651	0.590	0.645	0.633	0.759	0.693	0.645	0.687	0.723	0.723	0.699	0.693	0.675	0.645	0.717	0.681	0.723	0.729
O21	0.801	0.783	0.777	0.687	0.723	0.819	0.807	0.735	0.717	0.669	0.687	0.789	0.765	0.711	0.747	0.795	0.771	0.765	0.783	0.837	0.801	0.837	0.831	0.765
O22	0.747	0.608	0.687	0.789	0.741	0.741	0.717	0.633	0.675	0.627	0.741	0.711	0.699	0.681	0.729	0.729	0.717	0.699	0.669	0.651	0.759	0.711	0.705	0.771

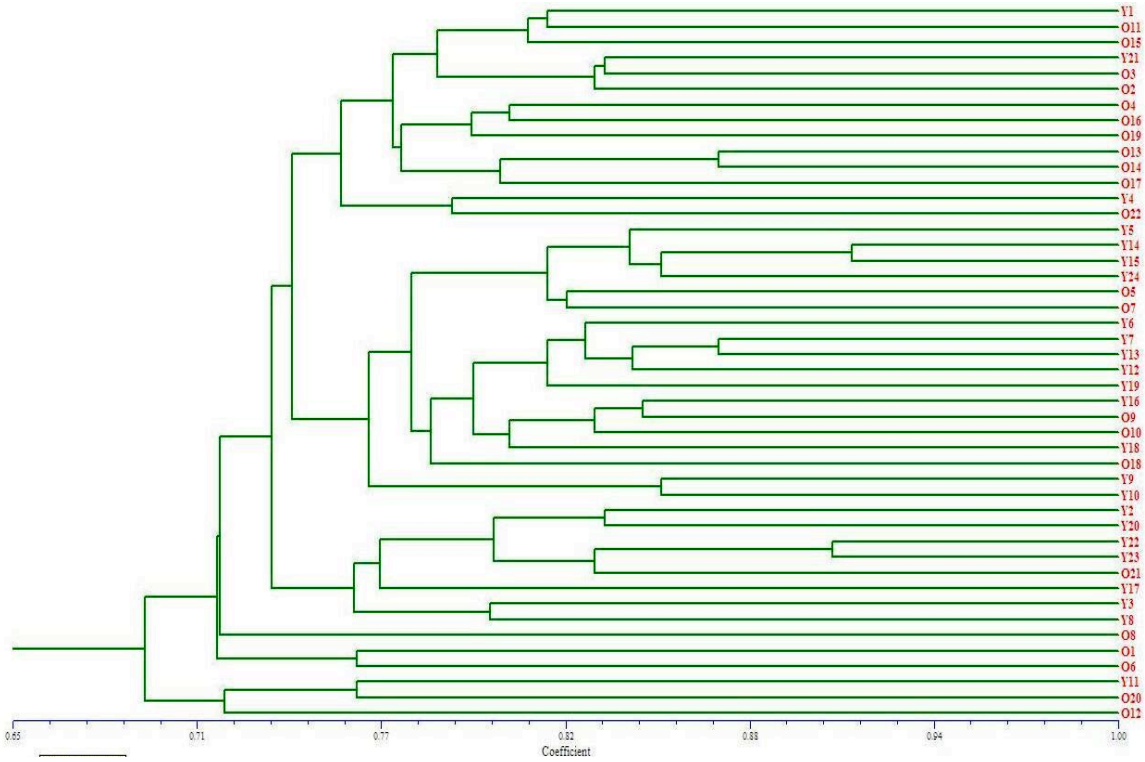

Figure1. UPGMA dendrogram for genetic relationship of investigated population.

DISCUSSION

The ISSR markers in this study yielded 139 polymorphic/166 total reproducible bands in 46 individuals which corresponded to two divided by age groups belong to the population of *P. vietnamensis* var. *fuscidiscus* in the Western North of Vietnam. This method provides a highly effective and reliable molecular-level tool for analysis of genetic diversity and genetic relationships within the variety.

This study reports the genetic diversity at population levels and also at individual groups which were classified by age. The extent of genetic variation within two different age groups of each population, the gene differentiation and the genetic distance among them were showed. Studied on the wide ranges of species which possessed the life history traits of dicotyledon, long-lived perennial life form, endemic, outcrossing breeding system and ingested seed dispersal mechanism which were also found in currently investigated variety, Hamrick and Godt (1996) reported that the genetic diversity based on allozyme were *PPB* = 42 – 46 %; H_e = 0.10 - 0.14; G_{ST} = 0.14 - 0.24, and Nymbom (2000) based on RAPD reported that the genetic diversity were H_e = 0.19 - 0.24; G_{ST} = 0.17 - 0.23. Thus, the results from this study showed that *P. vietnamensis* var. *fuscidiscus* in the Lai Chau province possessed high level of genetic diversity and the gene differentiation between young and mature individual groups was lightly small.

Achieved results showed the higher population genetic diversity related to *PPB* and heterozygousity than that has been reported in previous studies based on RAPD (Artyukova *et al.*, 2004), Allozyme (Jennifer *et al.*, 2004), and AFLP (Zhou *et al.*, 2005; Zhuravlev *et al.*, 2010) in other *Panax* populations. However, the similarity coefficients among the pair of samples in the current study were higher (Bai *et al*., 1997), which showed the limitations of ISSR markers in individual discrimination.

Using the same technique with current study to induce DNA fingerprinting in *P. ginseng* cultivated in North-East China, Li *et al.* (2011) reported that the genetic diversity was high at the species level (H_e = 0.2886; *PPB* = 98.96 %) but lower in cultivated types, viz. garden ginseng (h =0.2294, I = 0.3590, *PPB* = 85.42 %), forest ginseng (h = 0.1702, I =

0.2559, *PPB* = 57.29 %) and transplanted wild ginseng (*h* = 0.2021, *I* = 0.3125, *PPB* = 76.04 %). These parameters in separated populations of forest ginseng were ranged as *h* = 0.1065 – 0.1520; *I* = 0.2228 - 0.1854, *PPB* = 34.38 - 40.62 %). Genetic differentiation (G_{ST}) was also detected among divided by geographic locality subpopulations of forest ginseng 0.2328, garden ginseng 0.3187, and transplanted wild ginseng 0.2540. Comparing to these results, the current study revealed that genetic diversity of the naturally distributed *P. vietnamensis* var. *fuscidiscus* population in Western North of Vietnam was as high as compared to *P. ginseng* garden ginseng population, but it was higher than forest ginseng and transplanted wild ginseng populations in North-East China. The genetic differentiation among divided by age groups of population in this study was significantly lower than that among ginseng populations as previously investigated in the study from Li *et al.* (2011). The high level of genetic diversity in the study of Li *et al.* (2011) can be attributed to the investigated species' evolutionary development. Due to the long lifespan and overlapping generations of the populations within the prior study, considerable genetic variability has been accumulated and conserved under various selection traits during the evolutionary process (Li *et al.,* 2011) and this may happen to *P. vietnamensis* var. *fuscidiscus* population in Western North of Vietnam.

In Western North of Vietnam, near by the habitats of currently investigated population, there is the existence of other *Panax* taxon, namely *P. stipuleanatus* with two populations. The genetic diversity of this species was moderate at the species level (H_{eT} = 0.254; PPB_T = 96.02 %), and of its populations were lower as (H_{eBX} = 0.266; PPB_{BX} =91.48%) and (He_{HT} = 0.235; PPB_{HT} = 84.66 %) for BX and TH populations respectively, the interpopulation gene differentiation was G_{ST} = 0.03 with the genetic distance (D) among them was 0.0103 (Trieu *et al.*, 2016). The intergroup gene differentiation (G_{ST} = 0.0499) and the genetic distance between groups (*D* = 0.0298) were lower than the gene differentiation among subpopulation reported by Li *et al.* (2011). And it is easy to understand because these groups belong to the same population. However, the values of intergroup gene differentiation and the genetic distance between groups in current study were not small due to without geographic barrier. According to Vicente *et al.* (2003), the gene differentiation among populations from 0 to 0.05 is considered as small; from 0.05 to 0.15 is considered as moderate.

Because of the low gene differentiation and genetic distance, the two divided by age groups could not separate clearly in the UPGMA dendrogram of whole population (Fig. 1). Instead of this, most individuals belong to the same group had a tendency of grouping to form small clusters, which alternately arranged together. For genetic structure of investigated population, the young group possessed the lower genetic diversity (H_{eY} = 0.2086, I_Y = 0.3291, PPB_Y = 81.5 %) than mature group (H_{eO} = 0.2291, I_O = 0.3563, PPB_O = 84.34 %). And the intergroup gene differentiation among them was high as same as moderate for interpopulation gene differentiation in general as and even higher than *P. stipuleanatus* species reported in previous study by Trieu *et al.* (2016).

The similarity coefficient among mature individuals was more moderate (maximum = 0.873, minimum = 0.614 and average = 0.741) than that among young individuals (maximum = 0.916, minimum = 0.596 and average = 0.759). For total population, the maximum genetic similarity occurred in pair of young individuals of (Y_{14} – Y_{15}) and the minimum genetic similarity occurred between a young and a mature individual (Y_8 – O_{20}). These suggested that even possessed the high genetic diversity, the *P. vietnamensis* var. *fuscidiscus* population in Western North of Vietnam has coped with the risk of reduction in genetic diversity through generations. Studied on *P. quinquefolius* occurred from Georgia to West Virginia, Jennifer and Hamrick (2004) reported that the harvested populations possessed the higher genetic differentiation but lower expected heterozygosity compared to the protected populations due to harvest pressure.

In case of current study, there was a harvest pressure to investigated population as the same situation with *P. quinquefolius* ranged from Georgia to West Virginia. However, the genetic diversity of *P. vietnamensis* var. *fuscidscus* in Lai Chau - Vietnam was still high even as the risk of genetic reduction was evident, this showed that the geographic conditions where the investigated population occurs are suitable for its existence and development. In fact, recently a habitat of studied population has been narrowed by the climate change disaster and human unfavorable activities such as forest clearance, coal making, using the insecticides,

and animal hunting. These activities lead to the reduction of pollinators (insects) and dispersive animals (rodents and birds), changes of habitat, especially surface runoff.

The understanding on population genetic variability is essential to effective conservation and sustainable utilization. The relatively high genetic diversity at population and divided by age group levels are the advantages for conservation and development of *P. vietnamensis* var. *fuscidscus* in Western North of Vietnam. However, the number of discovered individuals was small, distribution area is narrow habitats, and the population showed the reduction in genetic diversity due to the human affects in the habitat and over-exploitation. Thus, the classification of this variety as critically endangered (CR) is suitable and worthy. Otherwise, because of the heavy harvest pressure, lacking of an actionable conservation strategy may lead to the increased reduction of genetic diversity and reserve of this variety. Thus, it is of critical importance to further investigate and protect this variety for conservation purposes and for sustainable development and harvest these valuable natural resources.

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ĐA DẠNG DI TRUYỀN QUẦN THỂ *PANAX VIETNAMENSIS* VAR. *FUSCIDISCUS* K. KOMATSU, S.ZHU & S.Q.CAI Ở TÂY BẮC VIỆT NAM BẰNG CHỈ THỊ PHÂN TỬ INTER SIMPLE SEQUENCE REPEAT

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TÓM TẮT

Panax vietnamensis var. *fuscidiscus* là loài dược liệu quý hiếm được tìm thấy ở huyện Sình Hồ và Mường Tè thuộc tỉnh Lai Châu, Việt Nam. Hiện nay, loài đang trong tình trạng bị đe dọa nghiêm trọng do khai thác quá mức và môi trường sống bị phá hủy. Thông tin đầy đủ về điều kiện sống và đa dạng di truyền làm cơ sở cho việc bảo tồn và phát triển. Trong nghiên cứu này, chỉ thị phân tử inter simple sequence repeat được sử dụng để khảo sát đa dạng di truyền và biến dị của 46 cá thể của quần thể phân bố tự nhiên ở Việt Nam. Đa dạng di truyền ở mức độ quần thể là cao (H_e = 0.2300, I = 0.3665 và PPB = 96.98 %). Nhóm cá thể trưởng thành có mức độ đa dạng di truyền cao (H_{eO} = 0.2291, I_O = 0.3563 và PPB_{O} = 84.34 %) so với nhóm cá thể có tuổi nhỏ (H_{eY} = 0.2086, I_y = 0.3291 và PPB_Y = 81.5 %). Biệt hóa di truyền trong quần thể cao (G_{ST} = 0.0499) với khoảng cách di truyền giữa các nhóm tuổi là 0.0298. Hệ số tương đồng giữa các cá thể của nhóm tuổi lớn là trung bình (Maximum = 0.873, Minimum = 0.614 và Average = 0.741) so với các cá thể ở nhóm tuổi nhỏ (Maximum = 0.916, Minimum = 0.596 và Average = 0.759). Dưới tác động của con người khai thác và phá môi trường sống, số lượng cá thể của loài rất ít, vùng phân bố rất hẹp, khai thác quá mức cũng dẫn đến làm suy giảm da dạng di truyền. Kết quả về đa dạng di truyền và biến dị cho thấy có sự suy giảm mạnh, nên ưu tiên bảo vệ toàn vẹn khu phân bố của quần thể.

Keywords: *Đa dạng di truyền, inter simple sequences repeat, Panax vietnamensis* var. *fuscidiscus, Việt Nam*